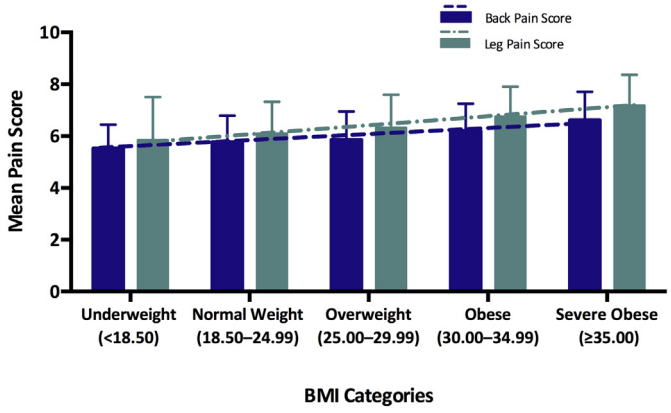
surgery, smoking status, work type, clinical diagnosis and relevant co-morbidities. Back and leg pain were modelled separately.

Results: The mean age of the study population was 50.9 years and the mean BMI was 27.2kg/m2; 54% of the patients were women. The mean back and leg pain scores were 6.2 and 6.7 respectively. With greater BMI, there was a linear increase in both back and leg pain (see figure). In our fully adjusted model, a 5-point increase in BMI was associated with an increase in back (0.15 units [95% CI 0.04,0.27]) and leg (0.22 units [95% CI 0.10,0.33]) pain scores. Female gender (0.36 units [95% CI 0.12,0.61]), heavy workload (0.65 units [95% CI 0.33,0.97]), rheumatoid arthritis (0.79 units [95% CI 0.40,1.18]), previous spine surgery (0.52 units [95% CI 0.26,0.79]), and depression (0.57 units [95% CI 0.42,0.71]) were all associated with increased back pain. These variables, as well as smoking (0.35 units [95% CI 0.08,0.61]), were significant predictors of leg pain.

Conclusions: In this large cross sectional study of spine patients presenting to tertiary European centres, several variables were found to predict higher pain scores. Obesity, as measured by increased body mass index, was associated with increased back and leg pain but, on account of the low coefficients, whether this increase is clinically meaningful is questionable. Nevertheless, weight loss could be a strategy for modulating back and leg pain; for instance, apart from its direct effects, it has been shown elsewhere that a high BMI is associated with depression, a strong predicator of back pain and leg pain. We also found that heavy workload and smoking were significantly associated with both back and leg pain, in agreement with earlier epidemiological studies. It has been suggested that these variables contribute to pain by inducing degeneration of the intervertebral disc. However, as we and others have shown, the association of these variables with disc degeneration is marginal hence our results suggest they could influence the experience of pain directly though the mechanisms involved still require identification.



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PRELIMINARY ANALYSIS OF THE CLINICAL PICTURE IN PATIENTS WITH SPONDYLOARTHRITIS DEPENDING ON THE TYPE AND SEVERITY OF CHANGES ON MRI

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Purpose: The aim of the study is to analyze the correlation between the type and severity of degenerative changes in the intervertebral disc in comparison to the incidence of neurological symptoms.

Methods: The study included 40 patients, 30 men and 10 women aged 52.2 ± 10.2 years. Mean index of BMI was 27.9±3.2 kg/m2. Each patient underwent neurological examinations with 1,5 T MRI of the cervical and lumbar spine.

Results: Prolaps of nucleus pulposus observed in each patient in mean 4 ± 2 vertebras. The most frequently was the second stage (76.4% of disc diseases, 92.5% of patients). Less frequently there were the third stage (37.5% of patients, 17.2% of disc diseases), the first (10% of patients, 4.5% of disc diseases) and the forth (2 patients, 1.9% of disc diseases) (table 1). Protrusion was observed significantly statistically frequently than bulging, extrusion and sequestration (1, 3 and 4stages: all p<0.0001), 3rd stage significantly statistically frequently than 1 (0.0039) and 4: (0.0004). The most frequently we observed deminishion of lateral recesses and intervertebral foramens : in 36 (90%) patients, compression on roots of spinal nerves: in 21 (52,5%) patients , and stenosis of spinal canal - in 9 (22,5%) pts, spondylolisthesis: in 5 (12,5%) pts. Spondylolisthesis and stenosis of spinal canal observed only in stage 2 and 3. Deminishion of lateral recesses and intervertebral foramens and compresion on roots of spinal nerves we observed significantly statistically frequently in stage 4 and 3 (88.9% and 55.6%, in both cases p<0,001). Deminishion of lateral recesses and intervertebral foramens were accompanied with 60% of disc diseases in stage 2 (protrusion) and 88.9% in stage 3 (extrusion). Compression of roots of spinal nerves we observed in 20% of cases and 55.6% of discopathy. Neurological deficits (motor and sensory) observed in 44.4% of patients with stenosis of spinal canal and 12.9% of patients without stenosis (p=0.0373). Neurological deficits significantly statistically frequently observed in 4th stage of discopathy (100%) than the other stages of patients (15.8%, p=0.0359).

Conclusions: Neurological deficits within patients observed with sequestration and stenosis of spinal canal in MRI examinations.

Table 1. The stages of disc diseases

The stages of disc diseases	Number of pts (n=40)	Number of discs (n=157)	Number of discs in relation to patients with discopathy
Bulging	4 (10.0%)	7(4.5%)	1.75
Protrusion	37(92.5%)	120(76.4%)	3.24
Extrusion	15(37.5%)	27(17.2%)	1.8
Sequestration	2(5%)	3(1.9%)	1.5
p	<0.0001	,0.0001	-

Table 2. Correlation of morphological changes in spine with neurological symptoms

Morphological changes in spine	Absent - A Present - P	Neurological deficits N=8	p
Spondylolisthesis	A - N=35 P - N=5	6 (17.1%) 2 (40%)	0.2568
Stenosis of spinal canal	A - N=31 P - N=9	4 (12.9%) 4 (44.4%)	0.0373
Deminishion of lateral recesses and intervertebral foramens	A - N=4 P - N=36	1 (25%) 7 (19.4%)	1.000
Compression of roots of spinal nerves	A - N=19 P - N=21	3 (15.8%) 5 ((23.8%)	0.6984
Bulging	A - N=36 P - N=4	8 (22.2%) 0	0.5658
Protrusion	A - N=3 P - N=37	0 8 (21.6%)	1.000
Extrusion	A - N=25 P - N=15	4 (16.0%) 4 (26.7%)	0.4439
Sequestration	A - N=38 P - N=2	6 (15.8%) 2 (100%)	0.0359

Stem/Progenitor Cells

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LOCALLY ADMINISTERED ADIPOSE DERIVED MESENCHYMAL STEM CELLS REINFORCE THEIR ANTI-INFLAMMATORY EFFECT THROUGH IL-1β MEDIATED ATTRACTION OF NEUTROPHILS INTO KNEE JOINTS WITH EXPERIMENTAL OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is characterized by cartilage breakdown and ectopic bone formation in joints. Recent studies have shown that low grade synovial inflammation, reflected by inflammatory factors like interleukin-1 beta (IL-1β), contributes to joint pathology. Recently we found that adipose derived mesenchymal stem cells (ASCs) exhibit anti-inflammatory characteristics and reduce joint pathology after local application into mouse knee joints with experimental OA. This anti-inflammatory effect is only observed after intra-articular injection in early but not late phase OA, suggesting that the effect may be mediated by pro-inflammatory mediators. Our objective is to study the effect of IL-1β on the anti-inflammatory potency of ASCs in early OA.

Methods: Experimental OA was induced by injection of collagenase into murine knee joints (CIOA). Total knee joints were stained with haematoxylin/eosin and the PMN specific antibody NIMPR14. ASCs were isolated from adipose tissue and stimulated with IL-1β or interferon-gamma (IFN-γ). Gene expression in synovium and stimulated cells were analyzed using qPCR. Protein levels of chemokines and cytokines were

measured in the supernatant and washouts using Luminex. ASCs were co-cultured with MACS isolated bone marrow (BM-) PMNs and analyzed using histology, qPCR and Luminex.
Results: Injection of ASCs into day 7 CIOA knee joints (when synovitis is highest) caused a strong influx of immune cells into the joint cavity shortly after injection (6 hours), which had largely disappeared after 24 hours. Immunohistochemistry revealed that particularly PMN-like cells were attracted. Synovial gene expression of neutrophil attracting chemokines KC, CXCL5, and CXCL7 was increased. In line with this, IL-1β stimulated ASCs injected in naive knee joints also resulted in massive influx of PMN-like cells. IL-1β and IFN-γ (as a positive control) stimulation of ASCs *in vitro* strongly enhanced gene expression of KC, CXCL5, and CXCL7 and protein levels of KC. Finally, we co-cultured ASCs with BM-PMNs in the presence of IL-1β or IFNγ. After 3 hours, a clear clustering of neutrophils around ASCs was observed which significantly decreased protein levels of KC (-69% after 24h; -76% after 48h).
Conclusions: ASCs attract PMN-like cells when injected locally into a day 7 CIOA knee joint expressing low levels of IL-1β. *In vitro*, IL-1β stimulated ASCs show an increase in chemokine expression, leading to attraction and clustering with neutrophils and significantly decreased levels of pro-inflammatory factors like KC. The anti-inflammatory effect of locally applied ASCs into OA joints showing synovitis may be triggered by IL-1β and attraction of PMN-like cells.

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THE CHARACTERIZATION AND FUNCTION OF ION CHANNELS IN SYNOVIAL FLUID DERIVED MESENCHYMAL PROGENITOR CELLS

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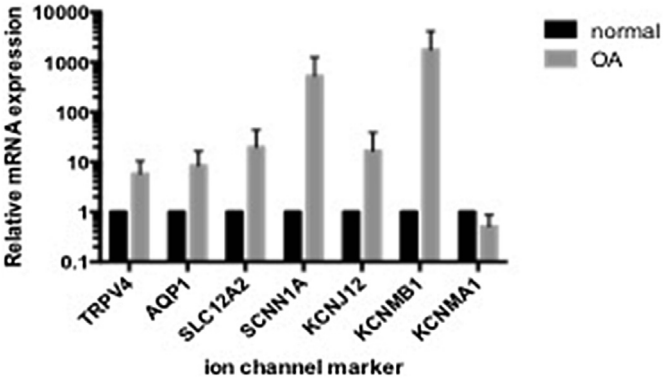
Purpose: In Osteoarthritis (OA) resident synovial fluid mesenchymal progenitor cells (sfMPCs) have greater proliferative ability but reduced chondrogenic capacity. One possible influence on the phenotype of these cells is the physiological environment of the joint. The osmolality of OA joints is significantly lower (~280mOsm) compared to healthy joints (~400mOsm). It was previously demonstrated that changes in osmolality can regulate the expression of chondrocyte gene expression, specifically Sox9. However, it is yet unknown if changes in osmolality regulate the gene expression in sfMPCs, and by extension, chondrogenesis of this cell population. The objective of this study was to determine the response of sfMPCs to changes in environmental osmolality conditions during chondrogenesis and by extension, if differential ion channel expression and function in normal and OA sfMPCs is the mechanism by which regulation of osmolality may occur.
Methods: Synovial fluid samples were collected from normal, OA and RA human knee joints. The osmolality of the fluid was quantified and the derivation/differentiation media was modified to span a range of osmolalities (264-375 mOsm). Chondrogenesis was measured with Alcian blue staining of cultures in addition to quantitative PCR (qPCR) using probes to Sox9, ACAN and Col2A1. Gene expression of six ion channels (TRPV4, SCNN1a, SLC12a2, AQP1, KCNMa1-KCNMb1 complex, KCNJ12) was studied in normal and OA sfMPCs using qPCR and flow cytometry was used to study the protein expression of TRPV4, KCNMb1, and KCNJ12 at the single cell level. A voltage clamp experiment was conducted to evaluate the functional potassium channels in both cell types.
Results: sfMPCs from arthritic joints demonstrated decreased chondrogenic potential compared to sfMPCs isolated from normal synovial fluid. Furthermore, the sfMPCs retained increased chondrogenic potential if differentiated under the same osmolality conditions for which they were initially derived within. Yet, with the change in osmotic environment the cell volume did not change in either case. qPCR results indicated that all ion channel genes examined, except KCNMa1, were upregulated in OA sfMPCs compared to normal.
However, patch clamp results demonstrated that OA sfMPCs showing detectable potassium inward rectifier current (54% vs. 79% in normal) and the amplitude of this current (measured at -80 mV) were significantly reduced compared to normal sfMPCs.
Flow cytometry results showed a significant decrease in TRPV4 in OA sfMPCs compared to normal cells, and normal vs. OA cells demonstrated a differential response to changes in extracellular calcium levels. Interestingly, however, the potassium inward rectifier channel (KCNJ12) and the calcium activated potassium channel (KCNMb1) demonstrated very little change between OA and normal cells.
Conclusions: Synovial fluid osmolality regulates the chondrogenic potential of normal and OA sfMPCs. Ions are regulated by both normal and OA sfMPCs through a combination of ion channels. These results suggest that there is differential ion channel regulation at the functional level, the protein level, and the gene level in OA and normal sfMPCs. Uncovering more about the regulation of ion channel proteins OA sfMPCs may uncover novel pharmaceutical targets for Osteoarthritis treatments in the future.



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HUMAN SYNOVIAL FLUID DERIVED MESENCHYMAL STEM CELLS EXPANDED UNDER LOW OXYGEN CONDITIONS AND IN A SERUM-FREE ENVIRONMENT EXHIBIT ENHANCED LINEAGE-SPECIFIC CHONDROGENIC POTENTIAL

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Purpose: Articular cartilage is an avascular tissue with a sparse chondrocyte cell population and has limited capacity for self-repair. The formation of lesions within cartilage, through injury or trauma, can initiate a degenerative process with the end result being osteoarthritis. Mesenchymal stem cells derived from the synovial fluid (SF-MSCs) of articulating joints have the ability to effectively form cartilage, and thus represent a potential candidate cell type for the development of cellular therapies aimed at repairing cartilage lesions. However, they can only be isolated in very small numbers, and thus, must be maintained in carefully controlled culture vessels that provide an environment conducive to rapid cell proliferation in order to generate clinically relevant quantities of cells. It is becoming increasingly apparent that oxygen tension in culture may play a very important role in both stem cell proliferation and differentiation. Moreover, the removal of animal-derived serum from the culture would facilitate the clinical translation of stem cell based therapies. Here the impact of oxygen level on the proliferation and differentiation potential of SF-MSCs under serum free conditions was evaluated.
Methods: Cell populations were isolated (under serum-free conditions) from human synovial fluid (collected from the non-osteoarthritic knee of patients) using established protocols, and characterized to verify the presence of SF-MSCs. Each isolated population was evaluated in a two-factor, two-level factorial manner (normoxic (21%) versus hypoxic (3%) oxygen levels; serum-containing versus serum-free medium). Cell growth kinetics, cell viability, cell morphology, and defining MSC characteristics were assessed for each condition to determine the most optimal for subsequent serial SF-MSC expansion in culture. Cells were grown in each condition and then exposed to either a standard osteogenic or adipogenic lineage protocol, or a chondrogenic differentiation protocol to assess which set of conditions was most conducive for chondrogenesis and subsequently evaluated qualitatively and quantitatively for cartilage characteristics. Statistical significance was evaluated using ANOVA.
Results: SF-MSCs propagated under hypoxic (3% oxygen) conditions, and in a serum-free environment displayed a higher average proliferation rate (doubling time = 21.73 h) compared to cells grown in serum-containing medium and normoxic (21% oxygen) conditions (doubling time = 55.45 h). Moreover, serum-free expanded cells were smaller in size and maintained a more round morphology compared to cells in serum-containing medium, which were bigger, and more